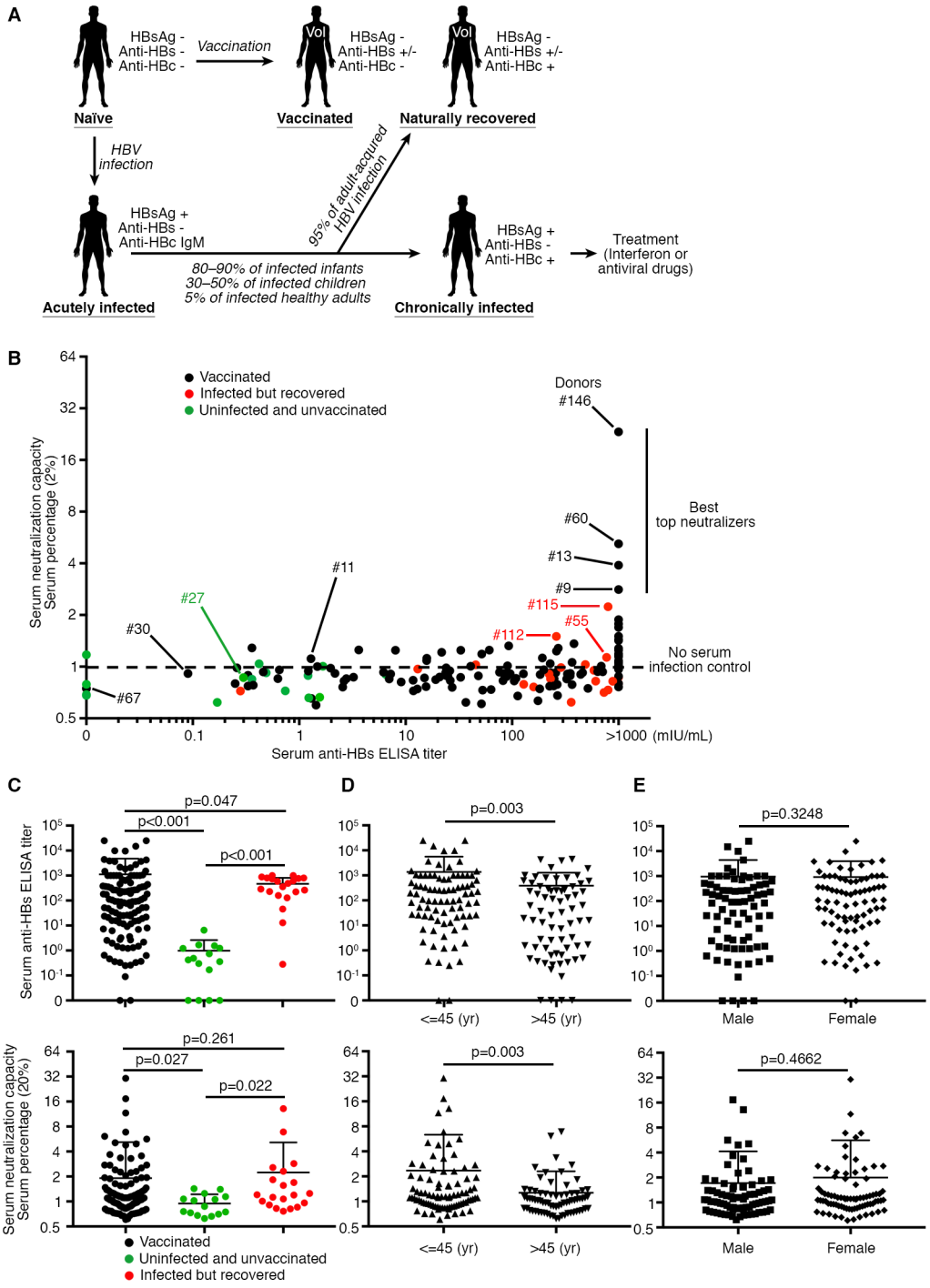


Figure S1. Characterization of Antibody Immune Response Against HBV, Related to Figure 1

(A) Schematic representation of different stages of HBV infection. Vaccinated or infected naturally recovered individuals were recruited for this study.

(B) Sera (1:50 dilution in the final assay volume) from 159 volunteers were screened, see also Figure 1A.

(C-E) Comparison of anti-HBs ELISA titers (upper panel) and their serum neutralization capacity (lower panel) between different groups of individuals. Vaccinated or recovered individuals show statistically higher anti-HBs titers (upper panel, C) and more potent neutralizing activity (lower panel, C) than the uninfected unvaccinated individuals. Younger individuals (≤45 years old) showed slightly higher antibody immune response against HBsAg (D). No difference was found between genders (E).

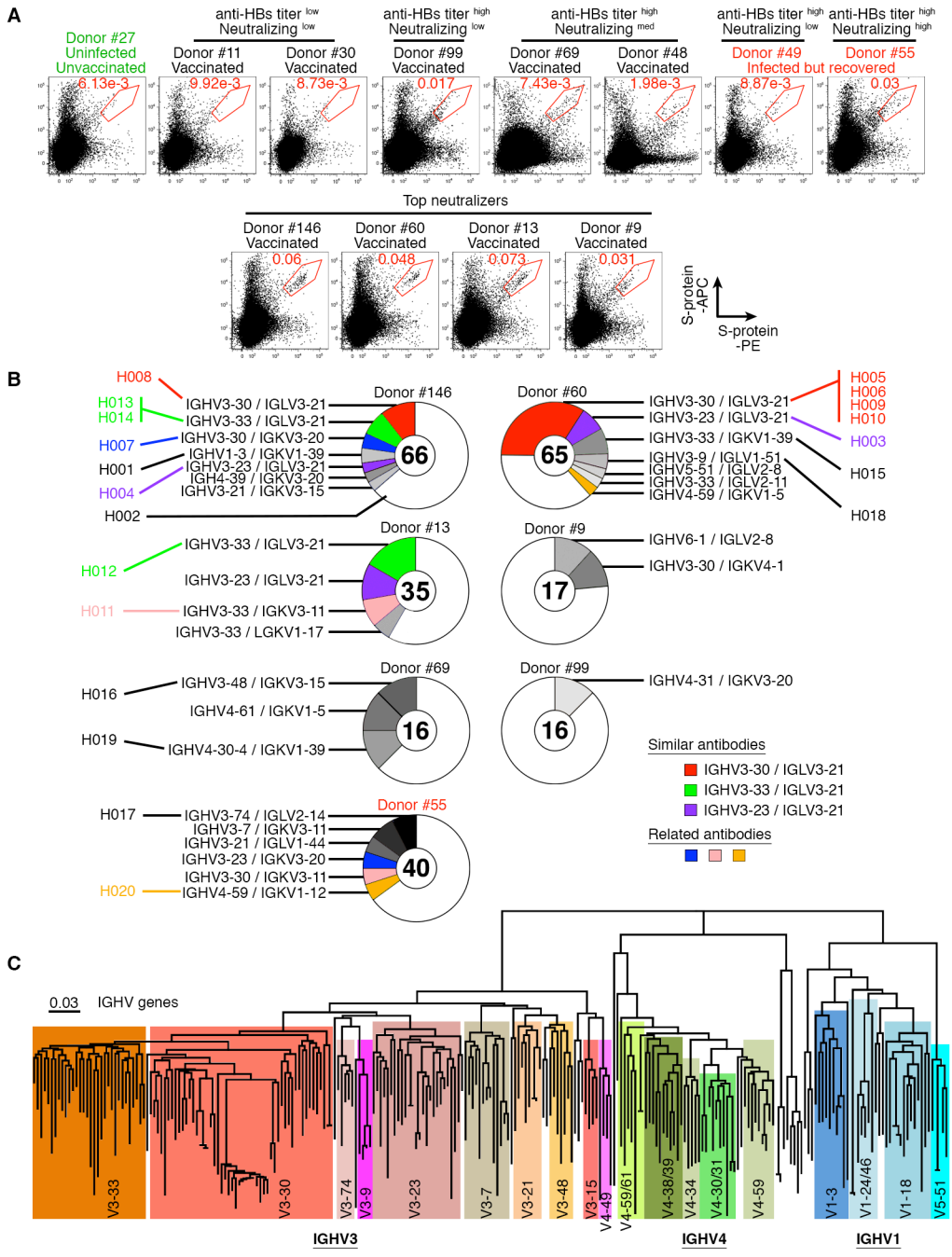


Figure S2. Antibody Cloning and Sequence Analysis of Anti-HBs, Related to Figure 2

(A) Frequency of S-protein-specific memory B cells in peripheral blood mononuclear cells of all twelve donors. Details are similar to Figure 2A.

(B) Pie charts show the distribution of anti-HBs antibodies. Figure legends are similar to Figure 2C. VH and VL genes for each slice are shown and the 20 chosen anti-HBs antibodies are labeled.

(C) Phylogenetic tree of all cloned anti-HBs antibodies based on IGH Fab region. IGH Fab regions from 244 memory B cells sorted with HBsAg were aligned followed by tree construction.

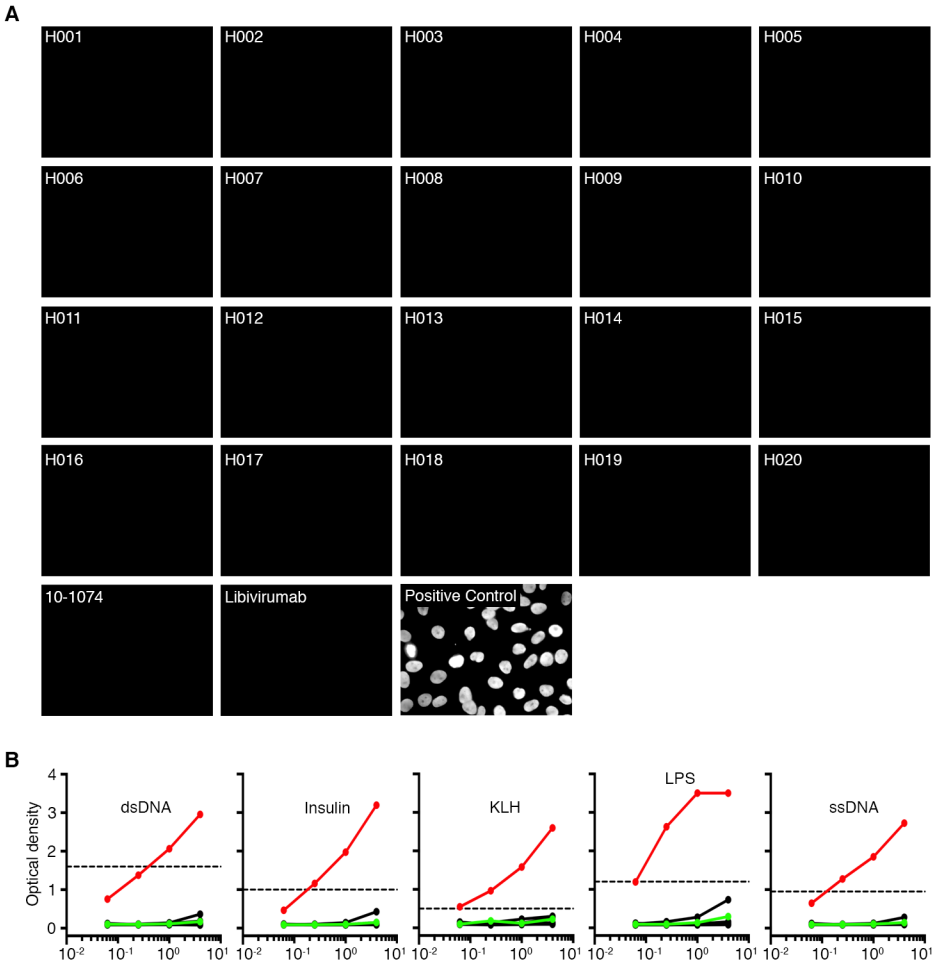


Figure S3. Autoreactivity of 20 anti-HBs antibodies, Related to Figure 3

(A) Autoreactivity of monoclonal antibodies. Positive control antibody efficiently stained the nucleus of HEp-2 cells. Twenty anti-HBs antibodies, as well as anti-HBs antibody libivirumab and anti-HIV antibody 10-1074, were also tested.

(B) Polyreactivity profiles of 20 anti-HBs antibodies. ELISA measures antibody binding to the following antigens: double-stranded DNA (dsDNA), insulin, keyhole limpet hemocyanin (KLH), lipopolysaccharides (LPS), and single-stranded DNA (ssDNA). Red and green lines represent positive control antibody ED38 and negative

control antibody mGO53 respectively, while dashed lines show cut-off values for positive reactivity (Gitlin et al., 2016).

A

	101 Q	G	M	L	P	V	C	P	L	110 I	P	G	S	T	T	T	S	T	G	120 P	C	K	123 T	C	T	
H020	123		128		98	133		121	107	42	95	110	108	106	98	105	102	93	93	95		111	102		103	Group-I
H004	96		96		79	105		100	96	71	113	101	103	103	101	100	100	101	92	72		97	108		112	
H006	97		87		79	106		92	110	65	114	102	97	97	98	96	98	95	92	96		102	99		103	
H019	112		115		100	122		105	106	54	112	99	92	92	99	91	99	95	114	48		75	98		83	
H017	57		69		68	60		87	85	106	100	80	91	90	91	88	98	89	84	104		98	82		78	Group-III
H007	99		86		129	85		108	85	90	99	105	103	104	107	105	99	107	102	76		97	31		97	Group-II
H016	98		109		116	81		94	96	110	86	97	103	102	104	103	106	109	113	95		61	25		95	
H015	75		79		99	68		80	100	78	80	85	94	95	94	100	96	100	94	114		93	94		108	

	131 T	P	A	Q	G	N	S	M	F	P	S	C	C	C	141 T	K	P	T	144 D	145 G	N	C	148 T	C	I	
H020	96	94		96	106	89	91	95	97	94	101				102	68	86	109	42	62	93		31		95	Group-I
H004	110	107		101	102	95	99	102	108	101	100				109	76	108	105	55	59	92		59		112	
H006	101	108		100	101	93	97	101	103	101	98				100	68	104	104	102	101	92		58		108	
H019	102	91		94	88	81	91	89	84	89	103				101	17	70	102	98	99	102		59		86	
H017	99	101		92	102	103	94	99	95	108	120				133	145	128	124	141	127	105		81		91	Group-III
H007	98	104		103	102	111	104	107	105	104	98				97	96	78	85	40	48	106		99		99	Group-II
H016	95	97		102	100	120	110	99	95	109	98				86	104	102	95	63	17	108		99		106	
H015	97	99		105	101	111	108	106	109	103	101				105	11	43	11	15	26	107		102		94	

	151 P	I	P	S	S	W	A	F	A	161 K	Y	L	W	E	W	A	S	V	R	F	171 S	W	Emp	WT	
H020	103		104	98	115			99		99	93	110	107	100	99		104	105		96	93	100		19	100	Group-I
H004	103		108	97	108			103		99	95	102	96	91	99		97	96		94	100	99		27	100	
H006	100		108	102	111			109		106	105	102	94	93	99		98	102		106	101	105		17	100	
H019	98		100	93	101			99		94	107	110	100	105	104		99	96		99	103	89		28	100	
H017	109		145	113	117			117		106	80	94	101	99	92		110	104		106	102	96		16	100	Group-III
H007	99		100	108	93			98		107	80	97	101	102	101		99	100		100	99	101		21	100	Group-II
H016	98		90	99	85			95		97	64	94	102	104	103		104	101		107	106	105		20	100	
H015	99		91	103	87			97		97	70	85	100	105	95		99	99		97	97	100		17	100	

B

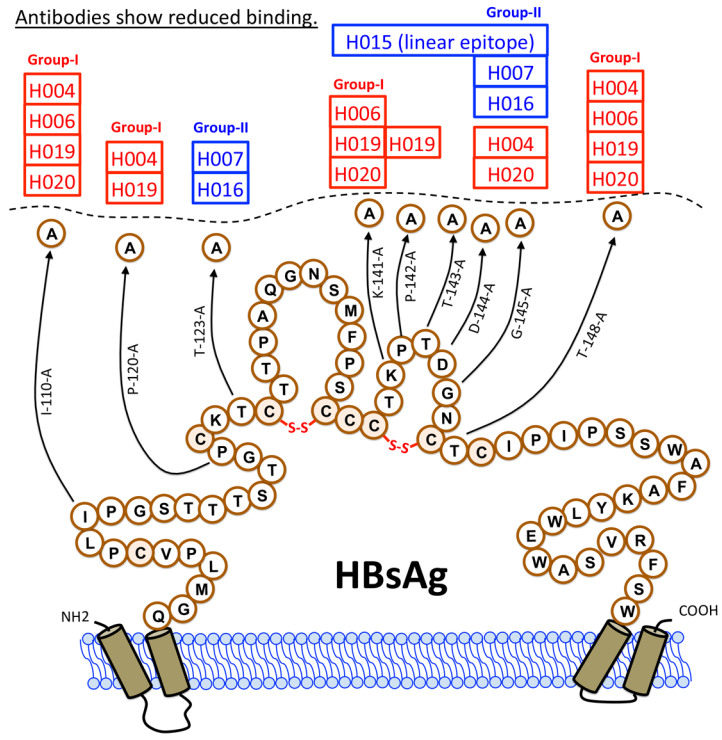


Figure S4. Alanine Scanning and Peptide Screening, Related to Figure 4

(A) Results of ELISA on alanine scanning mutants of HBsAg. Binding to mutants was normalized to wild-type S-protein: black, 0-25%; dark grey, 26-50%; light grey, 51-75%; white, >76%. Experiments were performed three times. Underlined cysteines, alanines, and amino acids known to be critical for S-protein production were not mutated (Salisse and Sureau, 2009).

(B) Schematic diagram of alanine scanning results.

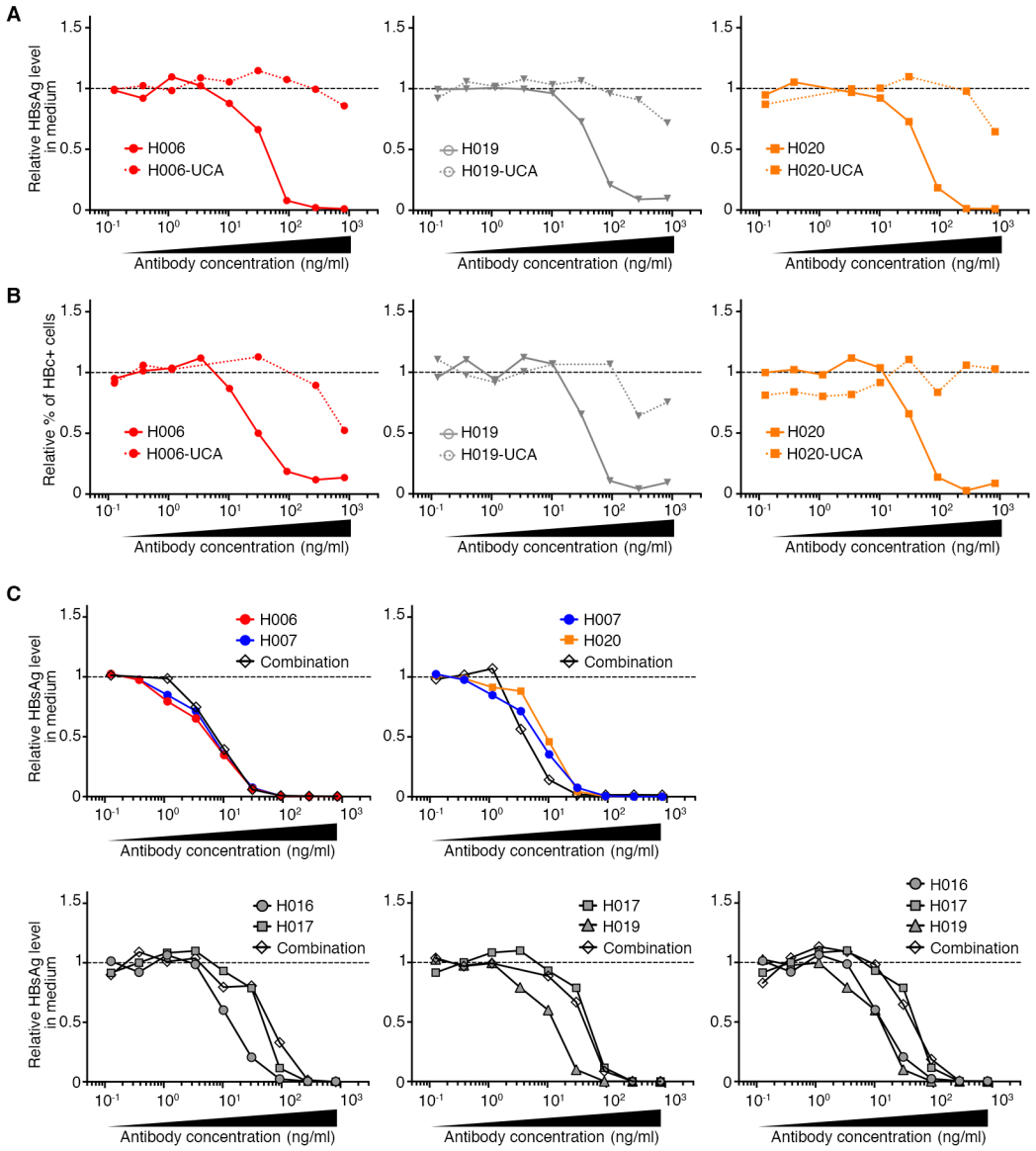
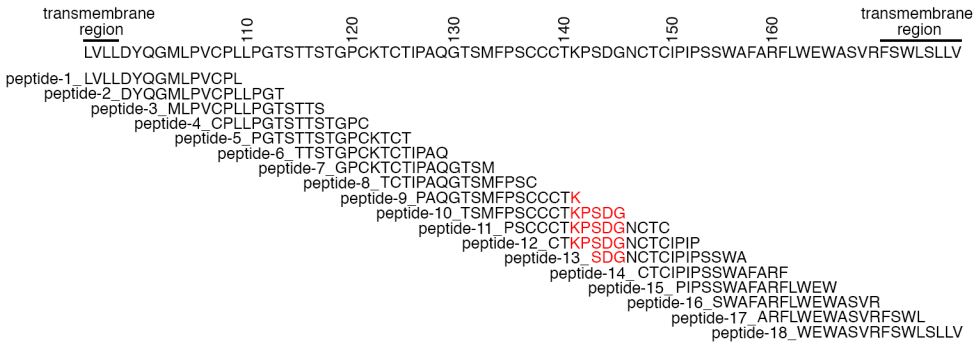


Figure S5. *In Vitro* Neutralization Assay of anti-HBs bNAb Unmutated Common Ancestor Antibodies or Combinations, Related to Figure 5

(A-B) *In vitro* neutralization assay of anti-HBs bNAbs and their corresponding unmutated common ancestor (UCA) antibodies. The relative infection rates were calculated based on either HBsAg protein level in culture medium (A) or HBcAg staining intracellularly (B).

(C) *In vitro* neutralization assay of anti-HBs bNAbs recognizing different epitopes and the same total amount of antibody combination at 1:1 or 1:1:1 ratio.

A

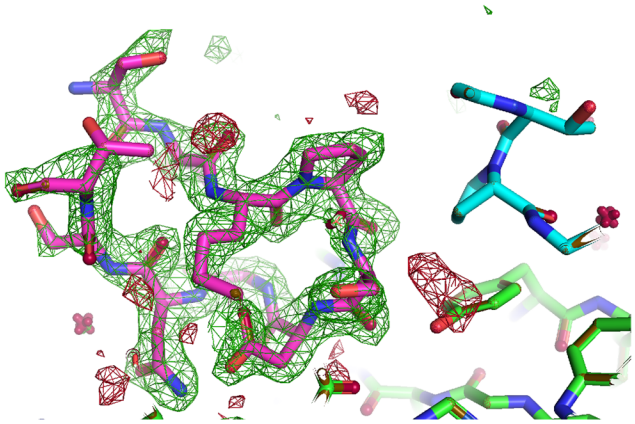


B

Wavelength (Å)	0.979
Resolution range	67.35-1.78 (1.82-1.78)
Space group	$P2_12_12_1$
Unit cell	49.9 71.2 134.7
Total reflections	615,285 (56,022)
Unique reflections	46,432 (4,386)
Multiplicity	12.9 (12.1)
Completeness (%)	99.5 (91.0)
Mean I/sigma (I)	17.2 (1.26)
Wilson B-factor	33
Anisotropy	0.449
R-merge	0.084 (1.26)
R-meas	0.090 (1.309)
R-pim	0.034 (0.505)
$CC_{1/2}$	0.999 (0.793)
Reflections used in refinement	46432 (4380)
Reflections used for R-free	1999 (188)
R-work	0.182 (0.276)
R-free	0.227 (0.293)

Number of non-hydrogen atoms	3752
Macromolecules	3391
Solvent	361
Protein residues	451
RMSZ (bonds)	0.37
RMSZ (angles)	0.55
Ramachandran favored (%)	98
Ramachandran allowed (%)	2
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.00
Clashscore	1.00
Average B-factor	39
Average B of H/L	39
Average B of the peptide	49
Macromolecules	38
Solvent	43
Fo,Fc correlation	0.97
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.32, 42.8

C



D

H-bonds within the peptide
Ser139(O) – Thr 148(N, OG1)
Lys141(N) – ASN146(O)
Lys141(O) – Gly145(N)
Asp144(OD2) – Asp146(N, ND2)
Asp144(OD1) – Asp146(N)
Salt bridge within the peptide
Lys141(NZ) – Asp144(OD2)
H-bonds between Fab and peptide
Lys141(NZ) – Asp H100(O)
Lys141(NZ) – Leu103(O)
Pro142(O) – Trp H52(NE1)
Ser143(O) – Tyr H59(OH)
Ser143(OG) – Glu H99(OE2)
Asp144(O) – Phe H53(N)
Salt Bridge between Fab and peptide
Lys141(NZ) – Glu H99(OE2)
Hydrophobic between Fab and peptide
Pro142 – Leu L29
Gly145 – Trp H52

Figure S6. Detailed Information of Crystal Structure of H015 and Its Linear Epitope, Related to Figure 6

(A) Synthesized peptides for antigenic loop region were subjected to ELISA for antibody binding. Among the tested antibodies, only H015 binds peptide-11 and -12.

(B) Data collection and refinement statistics for H015 Fab are summarized. Statistics for the highest-resolution shell are shown in parentheses. Refinement program PHENIX 1.16.

(C) The green/red density is the unbiased omit map. Red is negative density equated to noise.

(D) Table of contacts within the peptide and between Fab fragment and peptide.

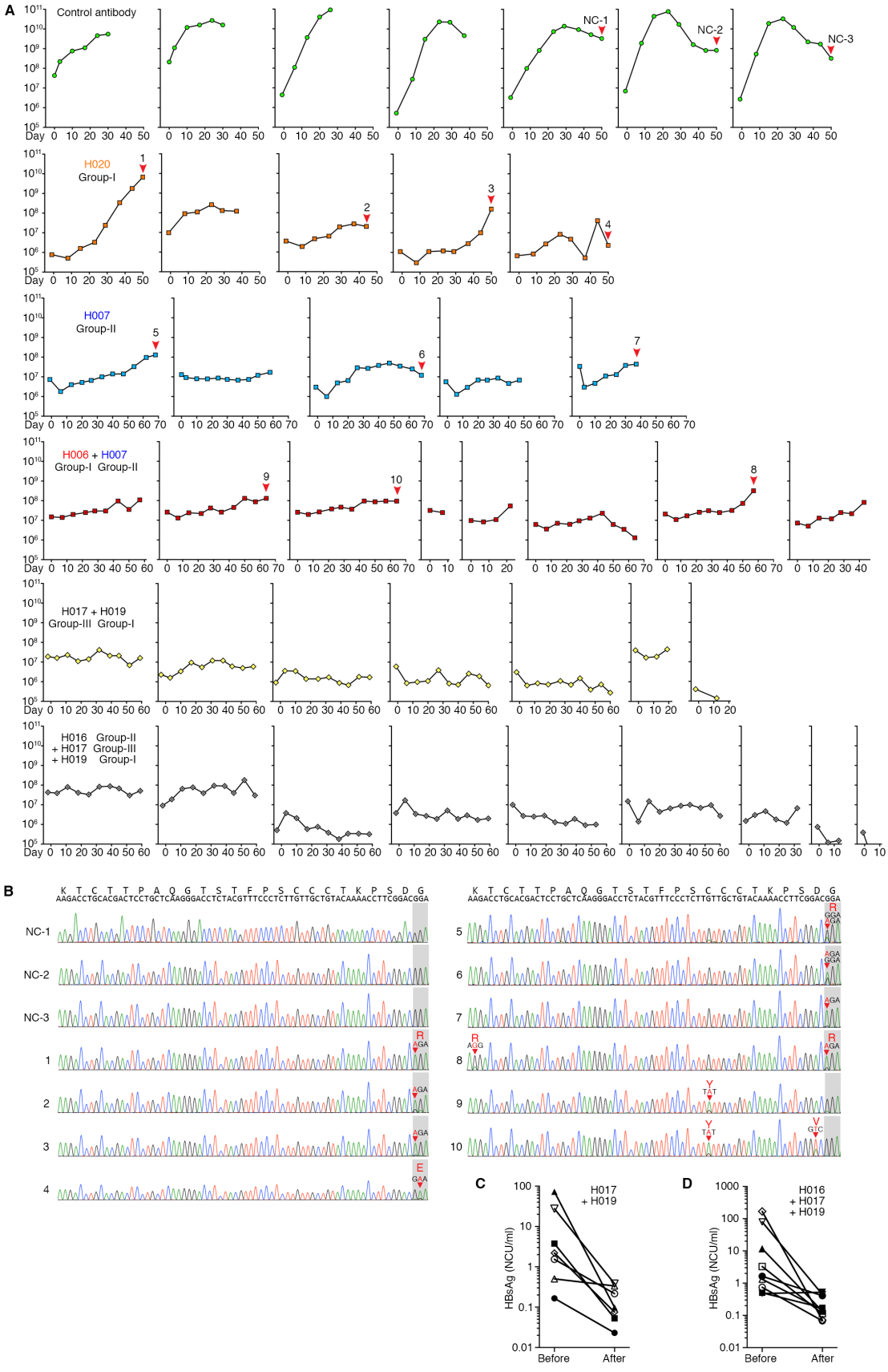

A
Control antibody
NC-1
NC-2
NC-3
H020
Group-I
H007
Group-II
H006 + H007
Group-I Group-II
H017 + H019
Group-III Group-I
H016 Group-II
+ H017 Group-III
+ H019 Group-I
Day
B
NC-1
NC-2
NC-3
C
H017
+ H019
HBsAg (NCU/ml)
Before
After
D
H016
+ H017
+ H019
HBsAg (NCU/ml)
Before
After

Figure S7. HBV DNA levels and S-protein Sequences in Antibody-Treated huFNRG Mice, Related to Figure 7

(A) HBV DNA levels in representative individual huFNRG mice treated by control antibody 10-1074, anti-HBs bNAb H020, anti-HBs bNAb H007, combination of anti-HBs bNAb (H006 + H007), (H017 + H019), and (H016 + H017 + H019). HBV DNA levels in mouse sera were monitored on a weekly basis. The mice without red arrows bear no escape mutations at the last time point.

(B) Part of the S-protein sequences from the indicated mice (red arrows and numbers) are shown below as chromatograms, with mutations marked by red arrowheads.

(C-D) HBsAg levels in mouse sera before and after antibody infusion. Mice were treated by anti-HBs combination H017 + H019 (C) (see Figure 7K) and H016 + H017 + H019 (D) (see Figure 7L). Each line represents a mouse with concentrations of serum HBsAg level expressed in NCU/ml (national clinical units per milliliter).